

Lab Updates

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Laboratories

Lyme Disease Western Blot

In October, 1994, the Second National Conference on Serologic Diagnosis of Lyme Disease [Centers for Disease Control and Prevention/Association of State and Public Health Laboratory Directors (CDC/ASTPHLD)] recommended a two-step testing system designed to standardize laboratory serologic testing for *B. burgdorferi*⁽¹⁾. Positive or equivocal results from a sensitive EIA or IFA (first step) should be further tested (supplemented) by a standardized Western Blot method (second step) for the qualitative detection of human IgM and IgG antibodies to individual proteins of *B. burgdorferi*.

Western Blot assays for antibodies to *B. burgdorferi* are considered supplemental rather than confirmatory because their specificity is less than optimal, particularly for detecting antibodies of the IgM class.

When testing specimens from patients during early *B. burgdorferi* infection, tests for IgM class reactivities are generally sensitive within the first two months after onset of symptoms, whereas tests for IgG class reactivities are more sensitive one month beyond onset of symptoms.

Structural and biological features of *B. burgdorferi* factor into which of the organism's proteins are most clinically and diagnostically relevant⁽²⁾. The organism is a Gram-negative spirochete, 0.2-0.25mm wide by 10-30mm long, that ultra-structurally resembles other spirochetes in the genus *Borrelia* with 7-11 periplasmic flagella that are attached subterminally to the protoplasmic cylinder and overlap in the center of the

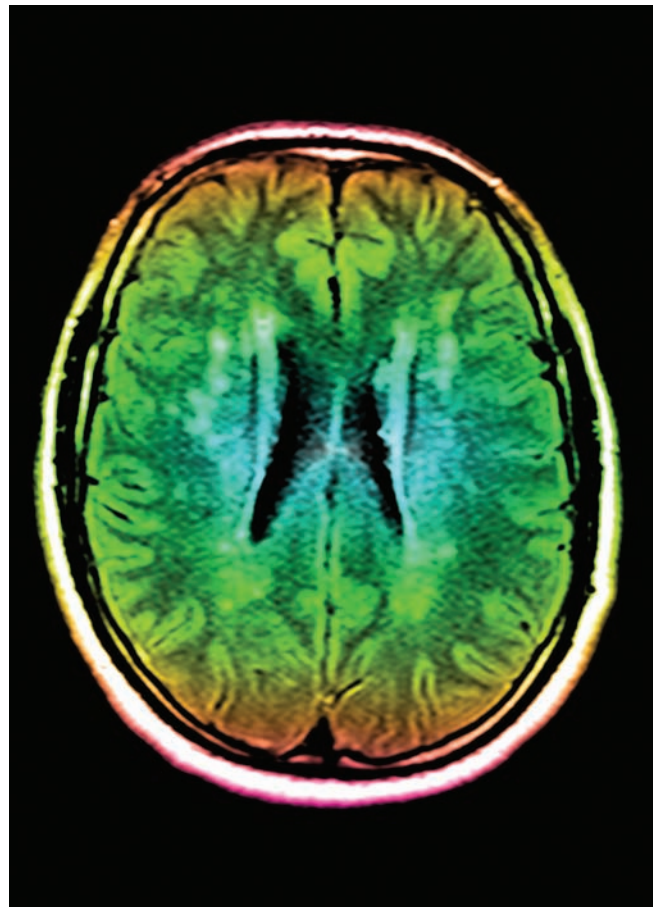


Photo: Kevin Vance

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cell. The main structural protein of the flagella is flagellin, a 41kDa protein. The outer membrane contains a number of outer surface proteins (Osp) that are anchored to the outer membrane by lipid moieties. These include OspA (31kDa), OspB (34kDa), and OspC (24kDa). Different Osp genes are expressed at different times during transmission from the tick vector (*Ixodes scapularis* in the eastern and midwestern United States) to the mammalian host. Another protein of the organism (60kDa), termed the “common antigen”, belongs to the heat-shock protein family. The RevA protein (~17kDa) is upregulated by temperature (as is OspC) and pH cues as the organism moves from the tick to the mammalian host⁽³⁾. The antigens highly specific for *B. burgdorferi* are the 21kDa (part of the OspC complex), 24kDa (OspC, to which IgM antibodies are frequently observed), 28kDa, 29kDa (OspD), 30kDa, 34kDa (OspB), 39kDa (antibodies mark early stage infection), and 93kDa (surface membranous vesicle; elicits primarily IgG antibodies in chronic stage infection).

The 31kDa antigen (OspA) is overexpressed, incurring non-specific reactivities, and thus cannot reliably be included with the highly specific antigens. Although the 41kDa antigen (flagellin) generates both IgM and IgG responses, it is not specific to *B. burgdorferi*. Neither the 60kDa (“common”) nor 66kDa antigens, both heat-shock proteins, are specific to *Borrelia* infections. However, either the 41 or 66kDa antigens can be diagnostically important if observed in conjunction with highly specific antigens.

Test Limitations

Sera from individuals with other spirochetal diseases such as syphilis, yaws, pinta, leptospirosis, relapsing fever and periodontal disease may yield false positive results. Individuals with other bacterial or viral infections, such as Rocky Mountain Spotted Fever, Epstein-Barr Virus, and cytomegalovirus may also have antibodies that cross-react with antibodies to *B. burgdorferi*. Individuals with connective tissue autoimmune diseases, such as rheumatoid arthritis or systemic lupus erythematosus, and individuals with anti-nuclear antibodies may also give false positive results. A negative Western Blot test pattern does not exclude the possibility of infection with *B. burgdorferi*.



Image Courtesy of Department of Health and Human Services
Public Health Image Library (PHIL)

References

- Centers for Disease Control and Prevention. Recommendations for test performance and interpretation from the Second National Conference on Serologic Diagnosis of Lyme Disease. *Morbidity and Mortality Weekly Report*. 1995;44:590-591.
- Shapiro ED, Gerber MA. Lyme disease. *Clin Infect Dis*. 2000;31:533-542.
- Carroll JA, El-Hage N, Miller JC, Babb K, Stevenson B. *Borrelia burgdorferi* RevA antigen is a surface-exposed outer membrane protein whose expression is regulated in response to environmental temperature and pH. *Infect Immun*. 2001;69:5286-5293

For any questions regarding methodology and interpretations, please contact:

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Changes in Serum and Urine Cortisol Testing



Cortisol (hydrocortisone) is the major glucocorticoid produced and secreted by the adrenal cortex. It affects the

- Metabolism of protein, fat, and carbohydrates
- Maintenance of muscle and myocardial integrity
- Suppression of inflammatory and allergic activities

Clinically measuring serum cortisol is useful for discrimination between primary and secondary adrenal insufficiency and in the differential diagnosis of Cushing syndrome. Cortisol secretion is episodic and the normal ranges are broad. Serum cortisol levels normally reflect that of ACTH & therefore have circadian rhythmicity. A single serum value, if it falls within the normal range, is inconclusive. An individual can have partial pituitary or adrenal insufficiency but maintain plasma ACTH and serum cortisol within the normal range. For these reasons, stimulation or suppression testing should be performed when there is doubt. Nevertheless, samples drawn at the appropriate time for the suspected endocrine dysfunction can be very helpful in excluding adrenal hypofunction or hyperfunction.

Cortisol bound to protein is protected from metabolism by the liver. Unbound (free) cortisol in serum is metabolized by the liver resulting in a wide variety of forms or metabolites. Many of these metabolites (conjugated, glucuronides and sulfate forms) are water soluble and rapidly voided in the urine. A small amount of cortisol (<50 ug/ 24 hours) and other extractable metabolites are also excreted in the urine.

Measurement of urinary free cortisol (UFC) provides a direct and reliable practical index of cortisol secretion. It is an integrated measure of serum free cortisol level that is not affected by body weight. 24-hour UFC, is increased in Cushing syndrome. This result is found in 95% of cases of Cushing syndrome. Values <50 g per 24 hours exclude the diagnosis. If basal UFC is more than $3 \times$ the upper limit of normal, confirms the diagnosis. If values are intermediate, a dexamethasone suppression test is indicated.

False-positive elevations of UFC may be seen in several conditions. High fluid intake significantly increases UFC. Any physiological or pathological condition that increases cortisol production raises UFC. Increased values may occur in depression, acute, chronic illnesses chronic alcoholism, eating disorders, and polycystic ovary syndrome and various drugs (e.g., carbamazepine, phenytoin, phenobarbital, primidone).

Therefore, in these conditions a normal result is more reliable than an abnormal one. False-negative results of urine cortisol collections also may occur. Because UFC reflects renal filtration, values are significantly lower in patients with moderate to severe renal impairment. A falsely low UFC can occur when creatinine clearance falls less than 60 ml/min, and UFC levels fall linearly with more severe renal failure. UFC can be normal if a patient has cyclic disease and collects urine when the disease is inactive. Finally, it may be normal in some patients with mild Cushing's syndrome, in whom salivary cortisol may be more useful.



It is important to ensure that patients provide a complete 24-h urine collection with appropriate total volume and urinary creatinine levels. The first morning void is discarded so that the collection begins with an empty bladder. All subsequent voids throughout the day and night should be included in the collection, which is kept refrigerated (but not frozen), up to and including the first morning void on the second day. Once the bladder has been emptied into the collection on the second morning, the sample is complete. Patients should be instructed not to drink excessive amounts of fluid and to avoid the use of any glucocorticoid preparations, including steroid containing skin or hemorrhoid creams, during the collection. Because UFC levels in a patient with Cushing's syndrome are variable, at least two collections should be performed, particularly in children in whom reproducibility can be low.



Effective April 11, 2011, following changes will be made to serum and urine cortisol testing.

New serum Cortisol AM reference range will be 6.7-22.6 mcg/dL. The current Cortisol AM range is 8.7-22.4 mcg/dL. There is no change to Cortisol PM Reference range (< 10 mcg/dL).

Urine Free Cortisol testing will be performed using highly sensitive and specific HPLC-Tandem Mass Spectrometry. The reference range is for Urine Free Cortisol is shown below.

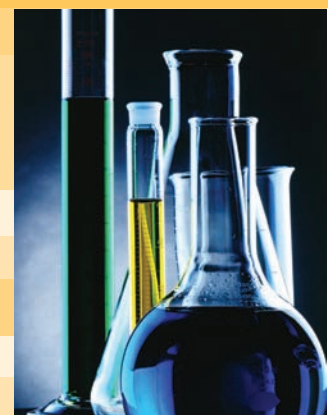


If you have questions, comments or suggestions, please contact:

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Reference Range for Urine Free Cortisol

Cortisol (µg/day)		
Males and Females:	3-8 years:	Less than 18 µg/day
	9-12 years:	Less than 37 µg/day
	13-17 years:	Less than 56 µg/day
Females:	18 years and older:	Less than 45 µg/day
Males:	18 years and older:	Less than 60 µg/day
Cortisol (µg/g creatinine)		
Females:	18 years and older:	Less than 45 µg/g creatinine
Males:	18 years and older:	Less than 32 µg/g crt



Changes to Carboxyhemoglobin (Carbon Monoxide, COHB) Testing



COHB is Hemoglobin (Hb) with carbon monoxide (CO) instead of the normal oxygen bound to it. CO has a much greater affinity than oxygen for Hb. The source of the CO may be exhaust (such as from a car, truck, boat or generator), smoke from a fire, or tobacco smoke. COHB is formed in CO poisoning. The COHB level is useful in judging the extent of CO toxicity and in considering the effect of smoking on the patient. A direct correlation has been claimed between CO level and symptoms of atherosclerotic diseases, angina, and MI.

COHB diminishes at a rate of about 15% per hour when the patient is removed from the contaminated environment. The most common cause of CO toxicity is exposure to automobile exhaust fumes. Significant levels of COHB can also be observed in heavy smokers. Victims of fires often show elevated levels from inhaling CO generated during combustion. Susceptibility to CO poisoning is increased in anemic persons.

Effective April 11, 2011, the following canned comment will be added to define the reference range variations between smokers and non smokers.

- Nonsmokers: 0.5–1.5 %
- Smokers (1–2 packs/day): 4–5%
- Heavy smokers (>2 packs/day): 8–9%

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Change in Insulin Reference Range

Insulin is a peptide hormone produced by beta cells in the pancreas. It regulates cellular uptake and utilization of glucose and is also involved in regulating carbohydrate and fat metabolism in the body. Type 1 diabetes is caused by insulin deficiency due to destruction of the insulin-producing beta cells. Type 2 diabetes is by far the most common type of diabetes and is characterized by variable degrees of insulin deficiency or resistance.

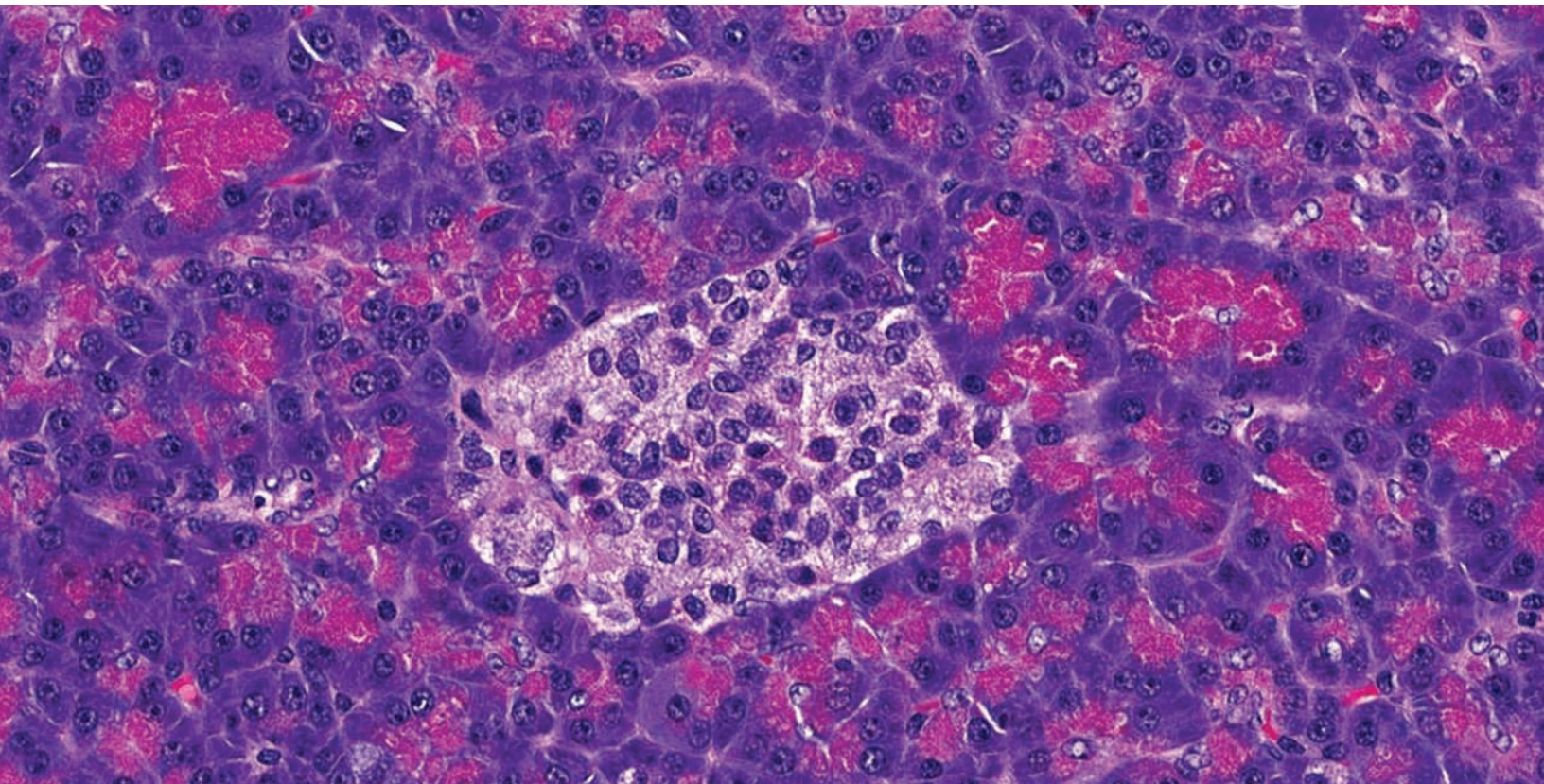
In normal individuals, serum insulin levels parallel glucose concentrations. As a result, concurrent measurement of insulin and glucose levels is necessary to evaluate insulin secretion properly. Fasting serum insulin concentration is typically higher in patients with type 2 diabetes than in healthy subjects, suggesting oversecretion of insulin in these patients. When obtained in the course of glucose tolerance test, insulin levels appear to be of some prognostic value in predicting the benefits of insulin therapy and the likelihood of progression to insulin-dependence. Insulin levels are also helpful in the diagnosis of pancreatic tumors secreting insulin. During prolonged fasting, when the patient's glucose level is below 40 mg/dL, elevated insulin level plus elevated levels of proinsulin and C-peptide suggest insulinoma.

Test	Current reference range	New reference range
Insulin	6 – 27 μ IU / mL	< 21 μ IU / mL

Effective March 28th, 2011, the reference range for serum insulin will change. This change is based on a recent reference range study that was done by the assay manufacturer and included 149 healthy subjects. There will be no changes in the methodology or sample requirements. It should be noted that the range applies to fasting samples only, therefore fasting serum samples are preferred. The range for non-fasting samples has not been established.

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This publication is made possible by Kevin Vance, Senior Director, Business Development and Marketing



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UMass Memorial Laboratories operates three laboratories in Worcester, Massachusetts, including a regional laboratory that is located in 38,000 square feet of state-of-the-art lab space in the Biotech Park, as well as laboratories at the University campus and Memorial campus of UMass Memorial Medical Center.